CLINICAL APPLICATIONS OF RETINAL OXIMETRY AND FLUORESCEIN ANGIOGRAPHY TO DIABETIC RETINOPATHY

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Honors Thesis
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May 2015

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ABSTRACT

Diabetic retinopathy (DR) is a disease caused by damage to retinal blood vessels as a result of chronic hyperglycemia, and thus has been linked to blood supply deprivation in certain retinal areas. My study aimed to determine whether quantitative analysis of retinal imaging methods could effectively evaluate DR patients. Retinal oximetry (RO), a new modality to determine relative retinal vessel oxyhemoglobin saturation, and intravenous fluorescein angiography (IVFA), which demonstrates patterns of retinal blood flow, were quantitatively analyzed for 27 healthy and 60 diabetic patients with a range of DR severity as determined qualitatively by clinicians. RO results showed a relationship between increased DR severity and higher oxygenation levels in retinal vessels, and IVFA results showed a similar correlation with increased DR severity and increased tissue ischemia. These results suggest decreased oxygen delivery to retinal tissue as DR severity increases. In the future, quantitative IVFA and RO may be used for risk stratification in DR diagnosis in asymptomatic patients at risk for vision loss from DR.

INTRODUCTION

Diabetic Retinopathy*:*

Diabetes mellitus (DM) is a disease in which a person has chronic hyperglycemia, which, over time, can cause microvascular damage in multiple organ systems including the retina. Diabetic retinopathy (DR) has four stages, the first three of which are categorized as *nonproliferative* diabetic retinopathy (NPDR). Microaneurysms form in mild NPDR, followed by blood vessel blockage in moderate NPDR, and greater vessel blockage and subsequent blood supply deprivation (ischemia) in severe NPDR. In the nonproliferative stages, as capillaries become damaged, vision loss may occur from macular edema (leaking

of fluid from blood into the center of the retina) or macular ischemia (loss of blood supply). The most advanced stage of DR, proliferative retinopathy (PDR), is marked by the growth of new, fragile, abnormal blood vessels on the retina, which can leak blood into the vitreous humor and lead to even more severely blurred vision.[1] Beginning in early stages of DR, endothelial damage causes blood vessel walls to weaken; some vessels dilate and other close. This leads to alterations in retinal blood flow, which subsequently affects oxygenation in the retina. DR is first treated by controlling diabetes systemically, that is, encouraging the patient to maintain normal blood glucose and blood pressure levels. NPDR without macular edema is not necessarily treated but is observed closely. NPDR must be identified early in order to minimize further disease development and adverse vision effects. To reduce the risk of vision loss, macular edema is treated with intravitreal anti-VEGF medications +/- focal laser surgery, and PDR is treated with scatter laser photocoagulation or vitrectomy surgery.[1]

***Retinal Oximetry**:*

Inner retina hypoxia as a result of blood supply deprivation has been linked to diabetic retinopathy in various studies.[2,3,4] It is further suggested that poor oxygen distribution in the retina of DR patients leads to high SO_2 (oxygen saturation) in retinal blood vessels.[2,5] However, constraints in non-invasive measurement techniques have limited the understanding of this relationship. As such, retinal oximetry may prove to be a valuable tool in evaluating the relationship between retinal oxygenation and DR by allowing precise quantitative measurements of retinal vessel oxygenation.

The retinal oximeter consists of a fundus camera with an attached image splitter, as well as a digital camera to record the image. The image splitter contains mirrors that split

the original beam from the fundus image into four smaller beams based on wavelengths of light, which are further filtered by different narrow band-pass filters in each of the four channels. In this study, two distinct wavelengths were used: one sensitive to oxyhemoglobin at 600 nm and one not sensitive to oxyhemoglobin at 570 nm.[6] Computer software (Oxymap Analyzer Software, version 2.3.2; Oxymap ehf., Reykjavik, Iceland) detects retinal vessels and uses relative light intensities inside and outside a vessel to calculate relative vessel oxygenation. The optical density (OD) of a vessel is a measure of the blood's light absorbance, calculated using a ratio of light intensity inside and outside of a vessel. The hemoglobin oxygen saturation (SO_2) of a vessel can be calculated using this information, as the optical density ratio (ODR) of ODs at specific wavelengths has been shown to have an approximately inverse linear relationship with SO_2. RO software color-codes the vessels and the rest of the fundus image based on an oxygen saturation scale. The software also allows the user to automatically select vessels in the fundus image, and aggregates pixel measurements to yield measurements for vessel SO_2, as well as data on oxygen partial pressure and vessel width.[7] RO thus allows for a non-invasive approach that can provide detailed information on retinal oxygenation, indicating significant diagnostic promise in the clinical setting for conditions that alter retinal oxygenation such as diabetic retinopathy, retinal vein occlusions,[8,9] and glaucoma[9,10].

Research on RO and DR is now underway, and early RO analysis of blood vessels indicates that patients with DR in general have higher vessel SO_2 levels than healthy subjects.[3,4,5,11] These results may indicate decreased oxygen delivery or decreased oxygen extraction in DR patients.[12] However, some studies have found that only venular SO_2 increases with DR,[2] and many do not address the change as the disease progresses. As

such, analysis correlating retinal vessel oxygenation with increasing severity of DR may yield further information towards understanding this relationship.

***Fluorescein Angiography**:*

Intravenous fluorescein angiography (IVFA) is a diagnostic tool used to visualize blood flow in the retina. The photographic equipment consists of a motorized fundus camera that can capture rapid sequential images, as well as an excitation filter. The capturing of images is preceded by the intravenous injection of a sodium fluorescein dye that becomes luminescent when excited by certain wavelengths of light (between 465 and 490 nm). The camera then captures fundus images in rapid sequence to allow the visualization of blood flow through the retina and choroid, with the excited dye indicating the flow of blood. Physicians can then use these images to visualize abnormalities in the progression of blood flow compared to that of a normal eye by looking for blockages in fluorescein flow, abnormal presence of fluorescein, or unusual concentrations or speeds of transmission of fluorescein.[13] IVFA is a current standard diagnostic imaging tool for diagnosing DR severity. The diagnostic utility of the IVFA is typically focused on observable microaneurysms, hemorrhages, and neovascularization in the fluorescent images.[14] IVFA has further been used to examine the pathogenesis of DR, demonstrating the phenomenon of capillary shunting around areas of non-perfusion.[15]

While IVFA has been primarily used as a qualitative diagnostic tool for DR, this study explored the potential use of quantitative information on retinal oxygenation.[16] IVFA shows retinal blood flow, and as such, ischemic areas, or lack of blood flow, are indicated by areas of non-perfusion by the fluorescein dye. Analyzing the ischemic ratio, defined as the area of ischemia divided by the total evaluated area of the retina, yields a

quantitative measurement for retinal perfusion and thus oxygenation.[17] This quantitative approach may yield more information on the relationship between DR severity and retinal oxygenation.

***My Study**:*

The study aims to further the understanding of the relationship between diabetic retinopathy and retinal oxygenation by quantifying changes in retinal oxygenation with DR disease stage using RO as well as quantitatively comparing retinal oxygenation and ischemia using IVFA. I hypothesize that there is a correlation between increasing severity of DR and both increasing retinal vessel oxygenation and increasing retinal tissue ischemia. This result would reflect the processes of thickened retinal vessel walls in diabetics, as well as retinal capillary shunting, in which capillary networks are circumvented and less oxygen is provided to tissue.[15] Both mechanisms would likely reduce the amount of oxygen delivered to retinal tissues, leading to hypoxia of the retinal tissue and hyperoxia of retinal vessels. Quantitative examination of oximetry among DR disease stages is vital because early RO studies have not yet fully addressed the relationship between increasing severity of DR and retinal vessel oxygenation. Additionally, in developing a standardized method for IVFA analysis of retinal tissue ischemia, the study will allow for a greater understanding of retinal blood flow and ischemia in relation to distinct stages of DR. Correlating RO and IVFA results will yield further information about whether retinal vessel hyperoxia and retinal tissue hypoxia are related processes as DR progresses. Understanding DR's effect on oxygenation and vasculature as the disease advances may lead to improvements in both diagnosis and risk stratification for DR.

METHODS

A) Subjects

Eighty-seven patients were enrolled in this study from the University of North Carolina (UNC) Department of Ophthalmology clinics. The study protocol was approved by the UNC Institutional Review Board, with 27 patients as healthy controls and 60 patients with Type I or Type II diabetes. Healthy patients were enrolled from the general ophthalmology clinic on routine check-ups. Diabetic patients were enrolled with the following clinical diagnoses: no DR (*n* = 16), mild NPDR (*n* = 6), moderate NPDR (*n* = 14), severe NPDR (n=7), or PDR (n=17). Inclusion criteria were: patients at least 18 years of age with Type I or Type II diabetes. Ophthalmologic exclusion criteria were: history of retinal vascular occlusions, glaucoma, macular degeneration, conditions with a media opacity such as dense cataracts or severe hemorrhaging that may obscure retinal photography, or prior vitrectomy or laser photocoagulation. Medical exclusion criteria were: severe respiratory disease (e.g. COPD), severe anemia, or sickle cell anemia. Exclusion criteria for diabetic patients that are contraindications for fluorescein dye (used in IVFA) were: cardiovascular event within 2 years, severe congestive heart failure, or chronic renal failure. Patients were enrolled after giving informed consent.

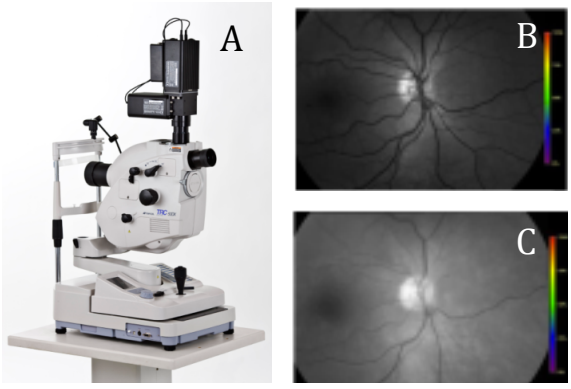

Figure 1. (A) The Oxymap T1 retinal oximeter (Oxymap T1 device connected to Topcon TRC50-DX fundus camera). (B) Image captured at 570 nm (isosbestic) and (C) image captured at 600 nm (sensitive to oxyhemoglobin).[18]

Retinal Oximetry

Image Acquisition:

Prior to image capture, patients' eyes were dilated with one drop each of 1%

tropicamide and 2.5% phenylephrine. Ophthalmic photographers obtained images of both eyes with the Oxymap T1 system (Oxymap T1 device connected to Topcon TRC50-DX fundus camera; Oxymap ehf., Reykjavik, Iceland) (**Figure 1**).[18] The device captures images at two distinct wavelengths, one sensitive to oxyhemoglobin (600 nm) and one isosbestic (570 nm), where the absorption spectra of oxyhemoglobin and hemoglobin cross. The images are each 1200 x 1600 pixels and cover a 50–degree angle of the retina, and were centered on the optic disc.

Image Processing:

For each patient, one eye was randomly selected for analysis. Images were analyzed with the Oxymap Analyzer software (version 2.3.2), which automatically detects vessels greater than 8 pixels in diameter, using the ring-method protocol previously established by this research group (**Figure 2**).[19]

The optic disc was excluded with a circle 250 pixels in diameter. Two additional circles with the same central point were applied with two and four times the diameter of the optic disc exclusion circle. Only the area between these two circles was used for analysis, with area within the inner circle excluded. Using the center of the optic disc as the central point, perpendicular lines were drawn to separate the image into four quadrants: superonasal (SN), inferonasal (IN), superotemporal (ST), and inferotemporal (IT). Exclusion of vessels beyond the diameter of the third circle and within the diameter of the second circle was implemented with a 31-pixel border. 19-pixel circles were as used to exclude certain areas where vessel detection would prove inaccurate (branching, overlapping, or intersecting), as well as segments of vessel less than 19 pixels in length.

Vessels to be measured were selected manually. Measurements were first taken to yield arteriolar SO_2 (SaO_2) by quadrant, starting with arterioles in the ST quadrant. Arterioles were subsequently selected in the IT, IN and SN quadrants, and then globally to measure the average SaO_2 for the image. These steps were repeated for venules to yield venular SO_2 (SvO_2) by quadrant and globally. As Oxymap software delivers measurements calibrated to healthy young individuals, results are relative to that calibration, occasionally allowing for SO_2 measurements greater than 100%. These values were not truncated to 100% as physiology would infer, per established oximetry protocol.[3,4]

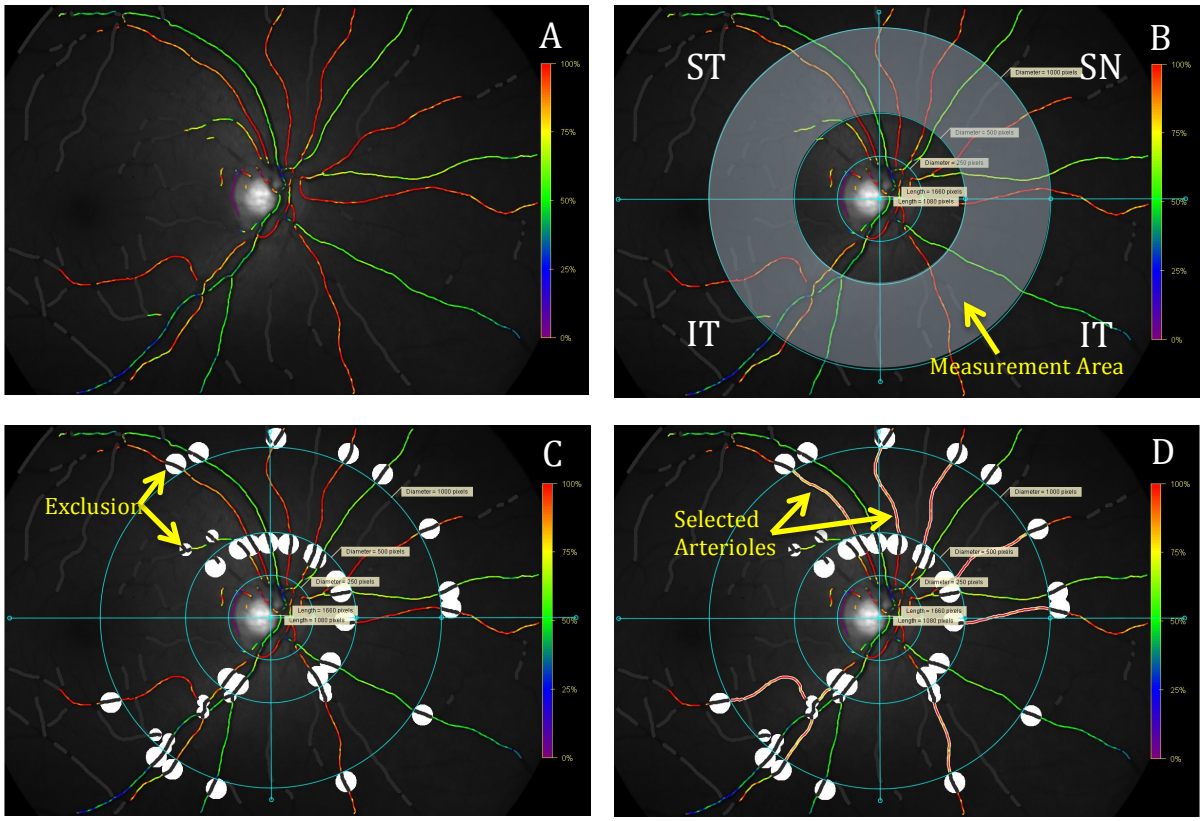

Figure 2. Ring method analysis of retinal oximetry images. (A) Image when opened with Oxymap software. (B) Quadrants and circle outlines in place. (C) Exclusionary areas mapped. (D) Global selection of arterioles.[19]

B) IV Fluorescein Angiogram

Image Acquisition:

DR patients were taken directly to the ultra-widefield scanning laser ophthalmoscope (Optos 200 Tx; Optos plc., Queensferry House, Scotland, UK) (**Figure 3**) following oximetry image capture. Color fundus photographs were obtained of both eyes. Sodium Fluorescein (5 cc) was injected intravenously by a physician. IVFA images were first captured on the transit eye indicated by the physician and the imaging sequence continued according to clinic protocol. In this study, the IVFA image with optimal perfusion of dye (about 40-50 seconds after injection) was used for analysis, and the corresponding color image was used to aid in detecting blood flow abnormalities.

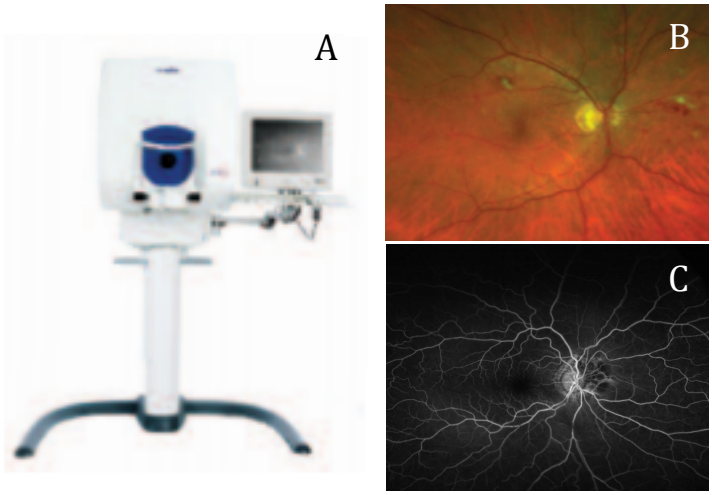

Figure 3. (A) The Optos 200 Tx device (for ultra-widefield fluorescein angiography). (B) Color fundus image and (C) Black-and-white fluorescein angiography image used for analysis.[20]

Image Processing:

For each patient, one eye was selected for analysis. This selection was the same eye analyzed for RO except in the case of image acquisition complications. In order to mask the analysis and increase objectivity, the selected analysis images and corresponding reference color fundus images were de-identified by a third party, unaware of the DR status of the patient and the RO results.

IVFA images were analyzed using ImageJ Software (Version 1.48v; U.S. NIH, Bethesda, Maryland) (**Figure 4**). Quadrant lines were transferred from RO analysis images. An ellipse was drawn to delineate the analysis area, capturing the optimal area of retina with dye perfusion (approximately 4.2 megapixel area). Pixel area measurements were obtained for the ellipse and each of the four quadrants. Ischemic areas were manually selected in each quadrant and area measurements were recorded.

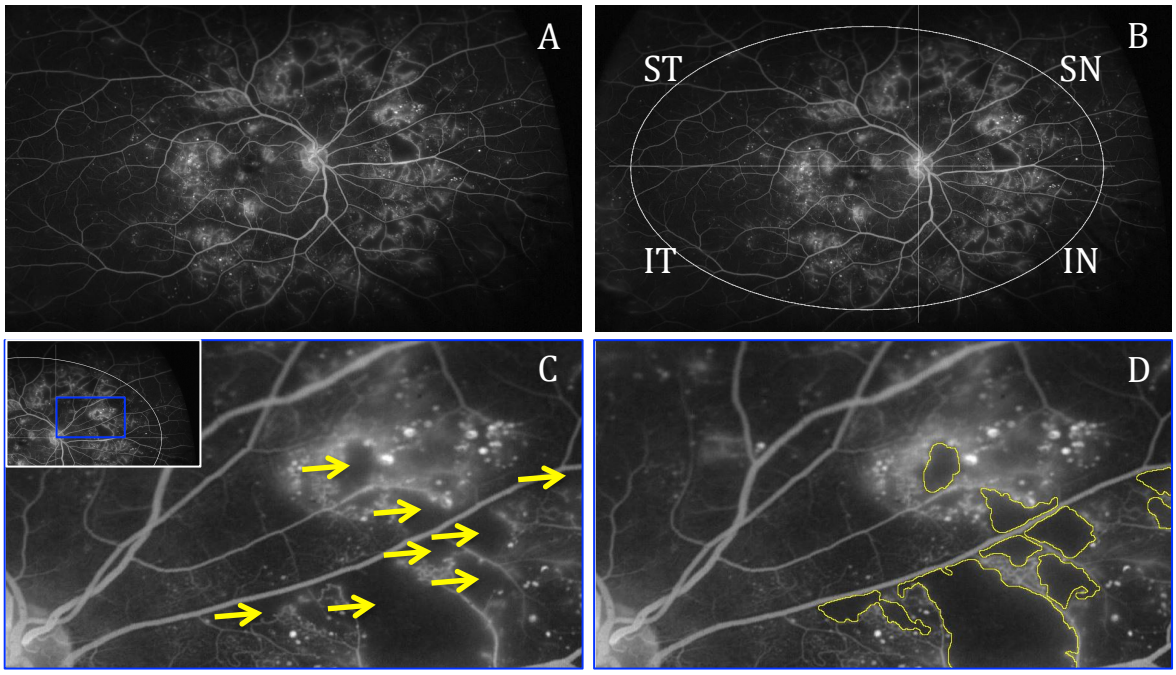

Figure 4. (A) Fluorescein angiogram image opened in ImageJ software. (B) Quadrants and ellipse in place. (C) Ischemic areas indicated with yellow arrows. (D) Selections of ischemic areas outlined in yellow for pixel measurement.

C) Statistical Analyses

Statistical analyses were implemented with SPSS software (version 20.0; SPSS, Inc., Chicago, IL). For retinal oximetry, one-way analysis of variance (ANOVA) was used to test the significance of difference in retinal oxygenation between each DR stage. This test was repeated for fluorescein angiography analysis results to test the significance of difference in ischemic ratio between each DR stage. Pearson's two-tailed correlation test was used to

assess the association between the two imaging methods by patient, correlating both SaO_2 and SvO_2 with ischemic ratio.

RESULTS

Demographic data for all study patients is displayed in **Table 1**, including group sample size, age, and marker of diabetes control (HbA1c).

Table 1. Demographic data for study patients.

Group		Value
Normal (n=27)		
	Age (years)	56 ± 10
	History of hypertension, # of patients (%)	8 (30)
DM w/o DR (n=16)		
	Age (years)	59 ± 9
	History of hypertension, # of patients (%)	15 (94)
	Hemoglobin A1c (%)	7.7 ± 2.4
Mild NPDR (n=6)		
	Age (years)	58 ± 19
	History of hypertension, # of patients (%)	4 (67)
	Hemoglobin A1c (%)	7.6 ± 1.2
Moderate NPDR (n=14)		
	Age (years)	53 ± 10
	History of hypertension, # of patients (%)	13 (93)
	Hemoglobin A1c (%)	8.5 ± 2.2
Severe NPDR (n=7)		
	Age (years)	53 ± 11
	History of hypertension, # of patients (%)	5 (83)
	Hemoglobin A1c (%)	8.1 ± 1.3
PDR (n=17)		
	Age (years)	52 ± 12
	History of hypertension, # of patients (%)	11 (78)
	Hemoglobin A1c (%)	8.3 ± 1.6

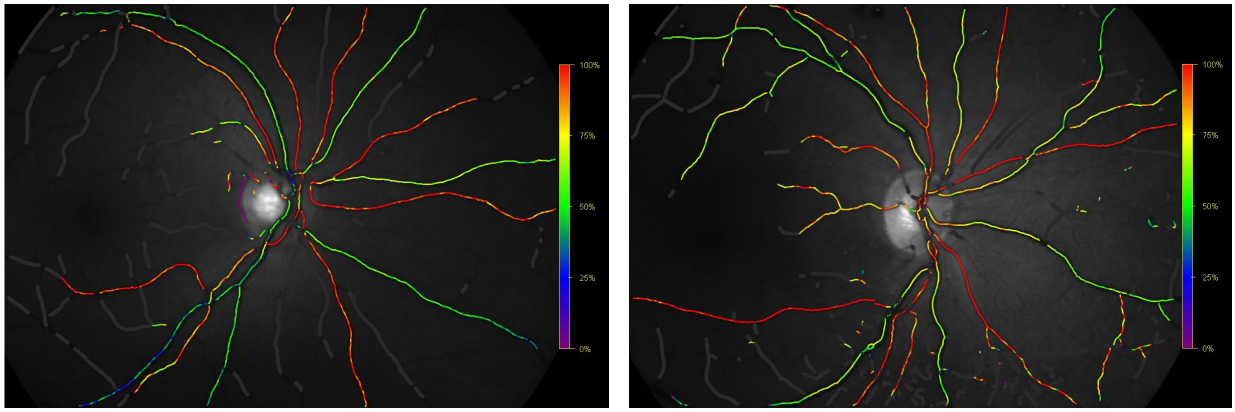

Figure 5. Oxymap images for (**A**) a healthy and (**B**) a PDR patient. As indicated by the oxygen saturation color scale, vessel oxygen saturation in the healthy patient is significantly lower than in the PDR patient.

Retinal oximetry images opened in Oxymap are displayed in **Figure 5** for a normal patient (**A**) and a PDR patient (**B**). Arterioles appear mostly red in both images, representing oxygen saturation of approximately 90-100%. The normal patient has many yellow areas interspersed, indicating oxygen saturation of approximately 75% and yielding a lower overall SaO_2. The difference in venular SO_2 is more apparent, with the normal patient showing a fairly consistent SO_2 of approximately 50% as indicated by green coloration (**A**). The PDR patient, in contrast, shows increased SvO_2, with coloration ranging from green to dark orange (approximately 50-95%) (**B**).

In general, global SaO_2 and SvO_2 values increased with increasing DR severity. **Table 2** summarizes global retinal vessel oxygenation values by DR stage. Global SaO_2 values tended to increase with increasing DR severity: normal 91 ± 4%; DM without DR 89 ± 8%; mild NPDR 92 ± 11%; moderate NPDR 93 ± 5%; severe NPDR 96 ± 14%; PDR 100 ± 7% (**Figure 6A**). Global SvO_2 values also tended to increase with increasing DR severity: normal 53 ± 6%; DM without DR 53 ± 10%; mild NPDR 53 ± 14%; moderate NPDR 62 ± 6%; severe NPDR 63 ± 13%; PDR 66 ± 11% (**Figure 6B**). Pair-wise ANOVA indicated a statistically significant increase in SaO_2 in PDR patients compared to normal patients

(p=0.003) and diabetic patients without DR (p=0.001). **Table 3** lists p-values for pair-wise comparison of SaO_2 and SvO_2 for all groups. By pair-wise ANOVA, there was also a statistically significant increase in SvO_2 in moderate NPDR patients compared to normal patients (p=0.038), as well as in PDR patients compared to normal patients (p<0.000) and diabetic patients without DR (p=0.004). Pair-wise ANOVA also yielded significant increase in the arteriovenous difference in moderate NPDR patients compared to normal patients (p=0.027); **Table 4** lists arteriovenous difference between all groups. However there was not a distinct trend in arteriovenous difference with increasing DR severity: normal 38 ± 5%; DM without DR 36 ± 6%; mild NPDR 39 ± 8%; moderate NPDR 31 ± 4%; severe NPDR 33 ± 11%; PDR 34 ± 11% (**Figure 6C**).

Table 2. Global oxygen saturation values (%) for retinal arterioles, venules, and the arteriovenous difference in oxygen saturation for all patients, given as mean ± s.d. (min to max).

Stage	Arterioles (SaO2)	Venules (SvO2)	Arteriovenous (A-V) difference
Normal (n=27)	91±4 (82.5 to 100.7)	53±6 (40.9 to 64.9)	38±5 (29.9 to 49.1)
DM w/o DR (n=16)	89±8 (66.8 to 102.0)	53±10 (23.4 to 65.0)	36±6 (24.4 to 43.6)
Mild NPDR (n=6)	92±11 (71.5 to 102.4)	53±14 (34.6 to 69.1)	39±8 (30.6 to 52.3)
Moderate NPDR (n=14)	93±5 (87.1 to 99.7)	62±6 (53.1 to 74.4)	31±4 (25.3 to 38.1)
Severe NPDR (n=7)	96±14 (77.0 to 124.5)	63±13 (40.9 to 76.5)	33±11 (21.3 to 48.4)
PDR (n=17)	100±7 (85.3 to 112.5)	66±11 (40.0 to 83.4)	34±11 (20.2 to 58.2)

Table 3. *P*-values for ANOVA pair-wise comparison using Tukey's post-hoc test for global venular oxygen saturation (SvO_2) and global arteriolar oxygen saturation difference (SaO_2) (*p < 0.05).

Stage	Normal	DM w/o DR	Mild NPDR	Mod NPDR	Sev NPDR	PDR
Normal		1.000	1.000	0.038*	0.126	0.000*
DM w/o DR	0.969		1.000	0.123	0.234	0.004*
Mild NPDR	1.000	0.973		0.367	0.425	0.059
Moderate NPDR	0.966	0.725	1.000		1.000	0.886
Severe NPDR	0.558	0.293	0.894	0.931		0.982
PDR	0.003*	0.001*	0.218	0.116	0.887	

Upper right: values for global venular oxygen saturation (SvO_2); lower left: values for global arteriolar oxygen saturation (SaO_2).

Table 4. *P*-values for ANOVA pair-wise comparison using Tukey's post-hoc test for global arteriovenous difference (A-V diff) (*p < 0.05).

Stage	Normal	DM w/o DR	Mild NPDR	Mod NPDR	Sev NPDR	PDR
Normal		0.886	1.000	0.027*	0.592	0.408
DM w/o DR			0.951	0.410	0.974	0.980
Mild NPDR				0.213	0.746	0.726
Moderate NPDR					0.973	0.815
Severe NPDR						1.000

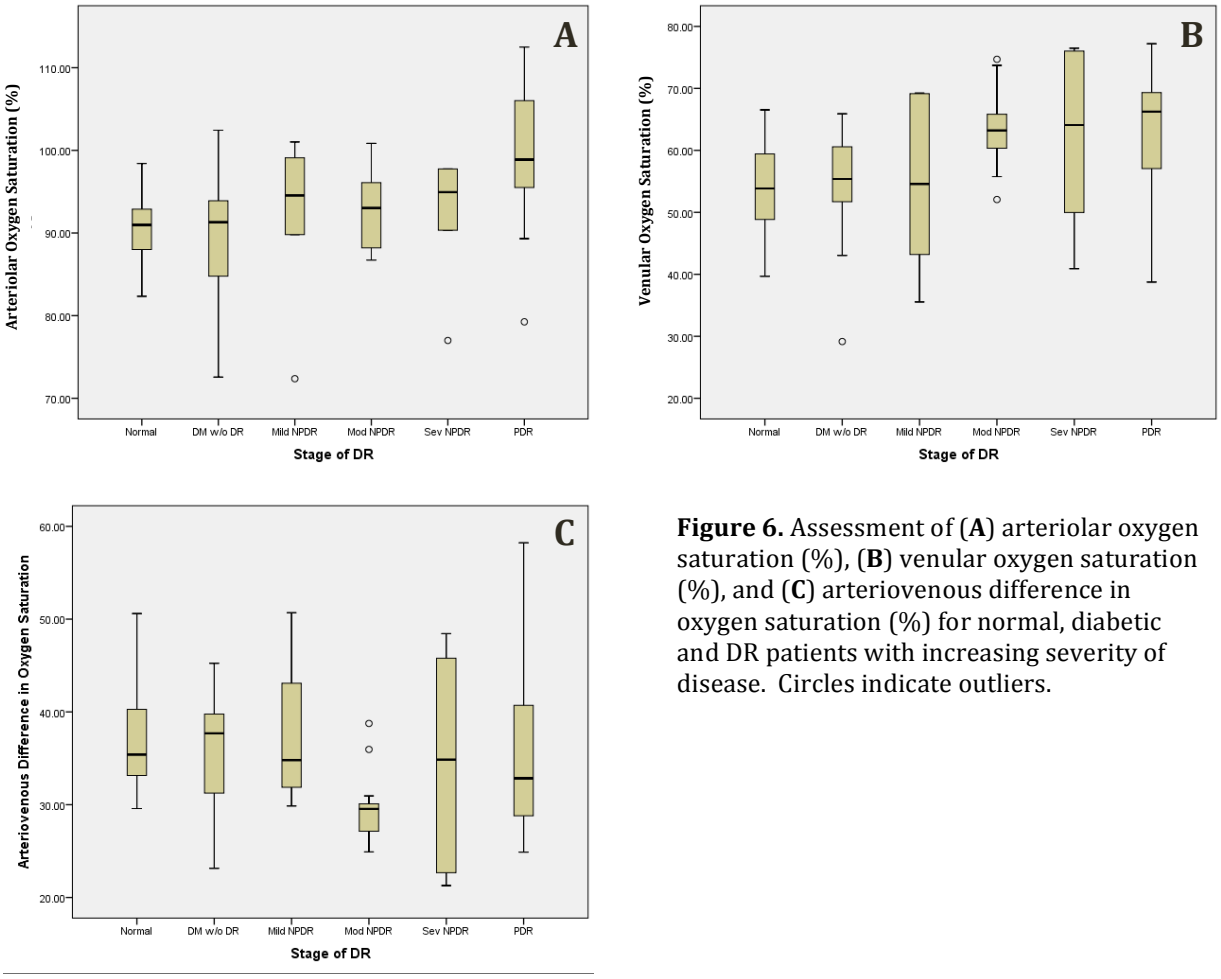

Figure 6. Assessment of (**A**) arteriolar oxygen saturation (%), (**B**) venular oxygen saturation (%), and (**C**) arteriovenous difference in oxygen saturation (%) for normal, diabetic and DR patients with increasing severity of disease. Circles indicate outliers.

IVFA images opened in ImageJ software are shown in **Figure 7** for a mild NPDR patient (**A**) and a PDR patient (**B**). The mild NPDR patient does not have any significant areas of ischemia or abnormalities in blood flow (**A**). The PDR patient has significant blood flow abnormalities, including noticeable vascular leakage (areas of white outside vessel

delineations in the image) and substantial ischemia (indicated in **B** with yellow arrows).

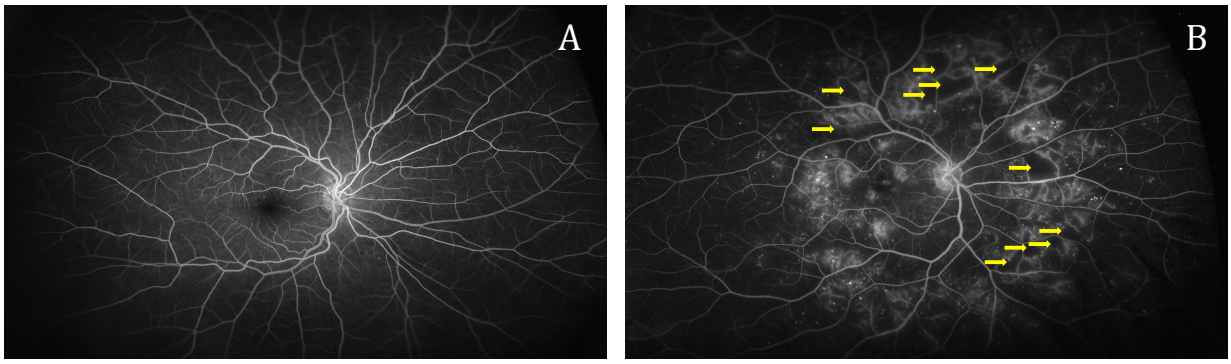

Figure 7. Fluorescein angiogram images for a (**A**) mild NPDR patient and (**B**) PDR patient. Yellow arrows indicate significant areas of ischemia in the PDR patient, in contrast with no ischemia in the mild NPDR patient.

Results from IVFA indicate that percentage of ischemic pixels increased with increasing DR stage: mild NPDR 0.76 ± 0.6%; moderate NPDR 2.21 ± 2%; severe NPDR 3.50 ± 2%; PDR 7.92 ± 9% (**Table 5**). This trend is displayed in **Figure 8**. However, pair-wise ANOVA did not yield significance in ischemia between any groups (**Table 6**). However, pairwise ANOVA did yield significance in percentage of ischemia between all NPDR patients (2.31 ± 2%) and PDR patients (7.92 ± 9%) (p=0.017).

Table 5. Global percentage of ischemic pixels in IVFA analysis of DR patients, given as mean ± s.d. (min to max).

Stage	Ischemic Pixels (%)
Mild NPDR (n=4)	0.76±0.6 (0.00 to 1.29)
Mod NPDR (n=9)	2.21±2 (0.21 to 7.81)
Sev NPDR (n=6)	3.50±2 (0.98 to 7.74)
All NPDR (n=19)	2.31±2 (0.00 to 7.81)
PDR (n=12)	7.92±9 (1.03 to 32.17)

Table 6. *P*-values for ANOVA pair-wise comparison using Tukey's post-hoc test for percentage of retinal ischemia ($p < 0.05$).

Stage	Mod NPDR	Sev NPDR	PDR
Mild NPDR	0.979	0.900	0.209
Mod NPDR		0.978	0.179
Sev NPDR			0.491
All NPDR			0.017*

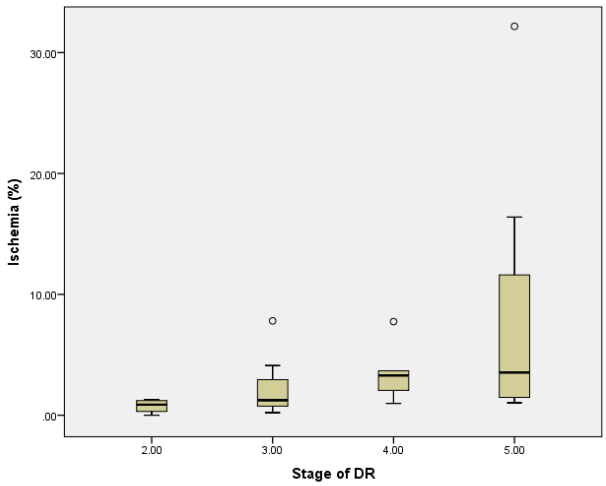

Figure 8. Assessment of tissue ischemia (%) for DR patients with increasing severity of disease. Circles indicate outliers.

The association between global ischemia and global venular oxygen saturation (A) and global arteriolar oxygen saturation (**B**) is displayed by scatterplot in **Figure 9**. Pearson two-tailed correlation analysis indicated significance in the positive correlation between global SaO_2 and percentage of ischemia (p=0.011). While not statistically significant, Pearson two-tailed correlation also indicated a slight positive correlation between global SvO_2 and percentage of ischemia (**Table 7**).

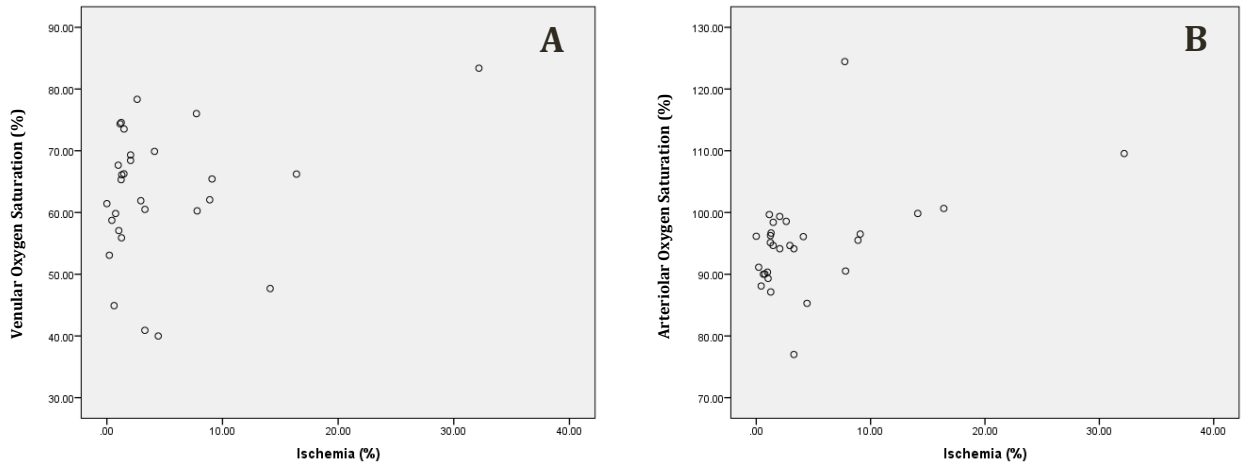

Figure 9. Scatterplot depicting the relationship of patients' tissue ischemia (%) and (**A**) venular oxygen saturation (%) and (**B**) arteriolar oxygen saturation (%) (n=29).

Table 7. Pearson correlation tests (2-tailed) for ischemia and venular oxygen saturation and ischemia and arteriolar oxygen saturation (*p < 0.05).

		Ischemia (%)
Global SaO_2	Pearson Correlation	0.467
	Sig. (2-tailed)	0.011*
Global SvO_2	Pearson Correlation	0.254
	Sig. (2-tailed)	0.184

DISCUSSION

Summary:

My study demonstrates that retinal oxygenation is altered in late stages of diabetic retinopathy. I found a significant increase in retinal venular oxygenation in moderate

NPDR and PDR patients compared to diabetics without retinopathy and to the normal controls. I also observed a significant increase in retinal arteriolar oxygenation in PDR patients compared to diabetics without retinopathy and to the normal control patients. Additionally, I found an increase in retinal tissue ischemia in PDR patients compared to NPDR patients.

***Retinal Oximetry and Diabetic Retinopathy**:*

Khoobehi et al.[4] reported a similar trend in retinal vessel oxygenation, showing increasing venous and arterial oxygenation with increasing severity of diabetic retinopathy. Khoobehi et al. observed increasing vessel oxygenation as disease severity increased, but only found significance in the comparison of healthy controls to severe NPDR and PDR groups. Similarly, I observed a similar trend with increasing disease severity, but only determined significance in the comparison of healthy controls and diabetics without retinopathy with PDR, as well as the comparison of healthy controls with moderate NPDR for venular oxygenation. The lack of significance in my comparison of healthy controls with the severe NPDR group for venular oxygenation may be due to the small sample size of the severe NPDR group (n=7). A substantial limiting factor in my study was patient sample size, and small sample sizes for mild NPDR (n=6) and severe NPDR (n=7) groups likely affected the lack of significance in oxygenation measurements when comparing those groups. For future analyses, increased sample sizes of all disease groups could allow better understanding of this relationship. My results are also similar to Bek[21], who observed significantly increased venous and arterial oxygenation with proliferative diabetic retinopathy. Jorgensen et al.[22] similarly saw an increase in venular and arteriolar oxygenation in PDR patients compared with healthy controls. However,

Jorgensen et al. did not observe a trend of increasing arteriolar oxygenation in non-proliferative stages of retinopathy, but rather a distinct increase in the proliferative stage. While not accounting for increasing severity of disease, Hardarson[11] and Stefannson[3] also found similar results, observing increased venular and arteriolar oxygenation with diabetic retinopathy compared to healthy controls.

Fluorescein Angiography and Diabetic Retinopathy:

While significantly less research has been done on correlating quantitative fluorescein angiography with retinopathy, Silva et al.[17] did find that angiography had substantial agreement with other diagnostic methods for determining severity of retinopathy, and that more lesions correlate with greater severity of DR. My study found a similar trend, observing increasing retinal tissue ischemia with increasing severity of DR. For this study, healthy and diabetic patients without retinopathy did not undergo fluorescein angiography due to the invasive nature of the imaging method. However, without healthy controls the study found significance only in its analyses between all NPDR groups and the PDR group, rather than between each stage. While control patients should exhibit no ischemia, including these patients could potentially give a more complete statistical analysis of progression of ischemia. Additionally, for the purpose of my study fluorescein angiography analysis was completed manually in ImageJ, and more consistency could be provided if ischemic areas were analyzed with an automated program.

Retinal Oximetry and Fluorescein Angiography:

The study found significance in the correlation between higher SaO_2 saturation and increased retinal tissue ischemia. However, I did not observe significance in the relationship between higher SvO_2 saturation and increased retinal tissue ischemia. These

results suggest the possibility of a weak correlation between analyses of retinal oximetry and fluorescein angiography, but significant further research would have to be undertaken to define that relationship. This relationship would likely reflect physiological mechanisms affecting blood flow that lead to both retinal tissue hypoxia and retinal vessel hyperoxia. For future studies, all patients should undergo both imaging methods to better correlate results.

***Physiological Explanations**:*

There are two major mechanisms that explain the increase in retinal vessel oxygenation and increase in retinal tissue ischemia in diabetic retinopathy: (1) capillary non-perfusion and shunting and (2) thickening of capillary vessel walls. Both of these mechanisms affect retinal blood flow, and thus the distribution of oxygen in the retina. In capillary shunting, some vessels dilate and others enlarge, leading to blood flow bypassing parts of the capillary network. Cogan and Kuwabara[15] observed that shunting occurs in diabetic retinopathy, and fluorescein angiography shows that enlarged capillaries shunt blood directly from retinal arterioles to retinal venules, bypassing retinal capillary networks. This leads to capillary non-perfusion, where blood is quickly transported through dilated capillaries, reducing the amount of oxygen provided to retinal tissues. This mechanism results in an unbalanced oxygen distribution, with hyperoxic retinal vessels and hypoxic retinal tissue. Secondly, thickening of capillary vessel walls may also decrease the amount of oxygen released to retinal tissue and increase the amount of oxygen retained in the blood. Ashton[23] and Roy et al.[24] have reported that capillary walls thicken with diabetic retinopathy, increasing the distance oxygen must diffuse from the bloodstream to reach retinal tissue. Capillary wall thickening may also explain hyperoxia of retinal vessels

and hypoxia of retinal tissue in diabetic retinopathy. Blood flow maldistribution and changes in oxygen extraction are the most likely explanation for my results, supporting the increase in arteriolar and venular oxygenation and increase in tissue ischemia in later stages of retinopathy.

CONCLUSION

This study found that retinal oxygenation is altered in later stages of diabetic retinopathy. There is a significant increase in retinal venular oxygenation in moderate NPDR and PDR patients, and an increase in arteriolar oxygenation in PDR patients compared to diabetics without retinopathy and to the normal controls. Ischemia of retinal tissue was also found to increase in PDR patients compared to NPDR patients. These results could reflect retinal capillary non-perfusion and thickened retinal vessel walls in diabetics, which reduce the amount of oxygen delivered to retinal tissues. This would match results of hypoxia of retinal tissue and hyperoxia of retinal venules as diabetic retinopathy progresses. For future studies, a larger sample size of mild and severe NPDR patients could help confirm the results and likely provide statistical significance between all DR stages. In the future, retinal oximetry may be used as an effective non-invasive tool to augment the diagnosis and risk stratification of diabetic retinopathy patients, and quantitative IVFA could provide standardization for existing qualitative IVFA studies.

ACKNOWLEDGEMENTS

I would like to thank my research sponsor, Dr. Seema Garg, for her guidance in the project. I would also like to extend thanks to the technicians, ophthalmic photographers, ophthalmology residents and research staff at the UNC Kittner Eye Center for their help and participation in the study, especially Sarah Moyer and Dr. Pooja Shah. I also would like

to express gratitude to my fellow research assistant this past summer, UNC medical student Abhi Guduru, as well as former UNC undergraduate Alexa Waters who set the foundation for the project in retinal oximetry. Further thanks to Dr. Tyson Hedrick, my Biology Department sponsor, who gave me significant guidance in project protocol, especially in the development of fluorescein angiography analysis. This research was also supported by a Summer Undergraduate Research Fellowship from the UNC Office of Undergraduate Research.

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